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SUPPLEMENTARY MATERIAL

STRUCTURE OF *ESCHERICHIA COLI* LYTIC TRANSGLYCOSYLASE MLTA WITH BOUND CHITOHEXAOSE: IMPLICATIONS FOR PEPTIDOGLYCAN BINDING AND CLEAVAGE*

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Tables S1 & S2, Figures S1 and S2

Table S1. B-factor analysis and glycosidic linkage conformations in the $(GlcNAc)_6$-bound sMltA-D308A tetramer

	Average B-factors[a], Å^2	Average dihedral angles of the β(1,4)-glycosidic linkages[b]	
		Φ, °	Ψ, °
GlcNAc -4	30.8		
		40.1 (±0.9)	2.2 (±0.4)
GlcNAc -3	25.4		
		37.5 (±0.7)	-11.0 (±0.7)
GlcNAc -2	25.1		
		48.4 (±1.0)	7.1 (±1.4)
GlcNAc -1	31.0		
		-18.4 (±4.4)	-50.3 (±4.2)
GlcNAc +1	39.8		
		48.1 (±14.2)	-3.0 (±10.8)
GlcNAc +2	52.5		

[a]Calculated per subsite and averaged for the equivalent GlcNAc residues in the four bound chitohexaoses.

[b]Dihedral angles Φ and Ψ are defined by bonds $H1_i$-$C1_i$-$O4_{i+1}$-$C4_{i+1}$ and bonds $C1_i$-$O4_{i+1}$-$C4_{i+1}$-$H4_{i+1}$, respectively. Values shown are the average values of the equivalent linkages in the four bound chitohexaoses. In brackets are the standard deviations. Favorable (Φ,Ψ) dihedral angles for the glycosidic linkages in chitin and peptidoglycan, as based on molecular dynamic simulations (1) and NMR analysis (2), are (~60º,~0º) and (~68º,~15º), respectively.

Table S2. Residues forming Protein-Sugar Contacts in Chitohexaose-bound sMltA-D308A

Subsite	Van der Waals contacts and hydrogen bonds[a]	Water or phosphate mediated hydrogen bonds
-4	Ser179 (sc,hb); Tyr180 (mc); Ala181 (mc); Lys237 (sc)	Lys237; Gln239
-3	Tyr180 (mc,sc,hb); Ala247 (sc); Val298 (sc)	Tyr180
-2	Tyr101 (sc); Gly163 (mc); Ser164 (sc,hb); Tyr180 (sc), Lys245 (mc); Gly246 (mc); Ala247 (mc,sc), Asp297 (hb), Val298 (sc,mc), Gly300 (mc)	Tyr180
-1	Tyr101 (sc), Val161 (mc,sc,hb), Gln162 (mc); Gly163 (mc); Tyr188 (sc); Ala259 (sc); Asp297 (sc); Val298 (mc); Gly299 (mc)	Gly300; Ala301
+1	Gly100 (mc,hb); Val161 (sc,mc,hb); Gln162 (sc,hb); Tyr188 (sc); Met208 (sc); Ala301 (sc); Ala321 (mc); Gly322 (mc)	
+2	Thr99 (sc), Ser190 (sc), Tyr324 (mc), Ala321 (mc) ; Gly322 (mc); Asn325 (sc,hb),	

[a]van der Waals contacts were identified with a 4.0 Å cutoff distance. For hydrogen bonds the acceptor and donor atoms lie between 2.5-3.4 Å apart with favourable geometry. The abbreviations used are: sc, van der Waals contact with side chain; mc, van der Waals contact with main chain; hb, hydrogen bond. Underlined residues are strictly conserved among different MltAs.

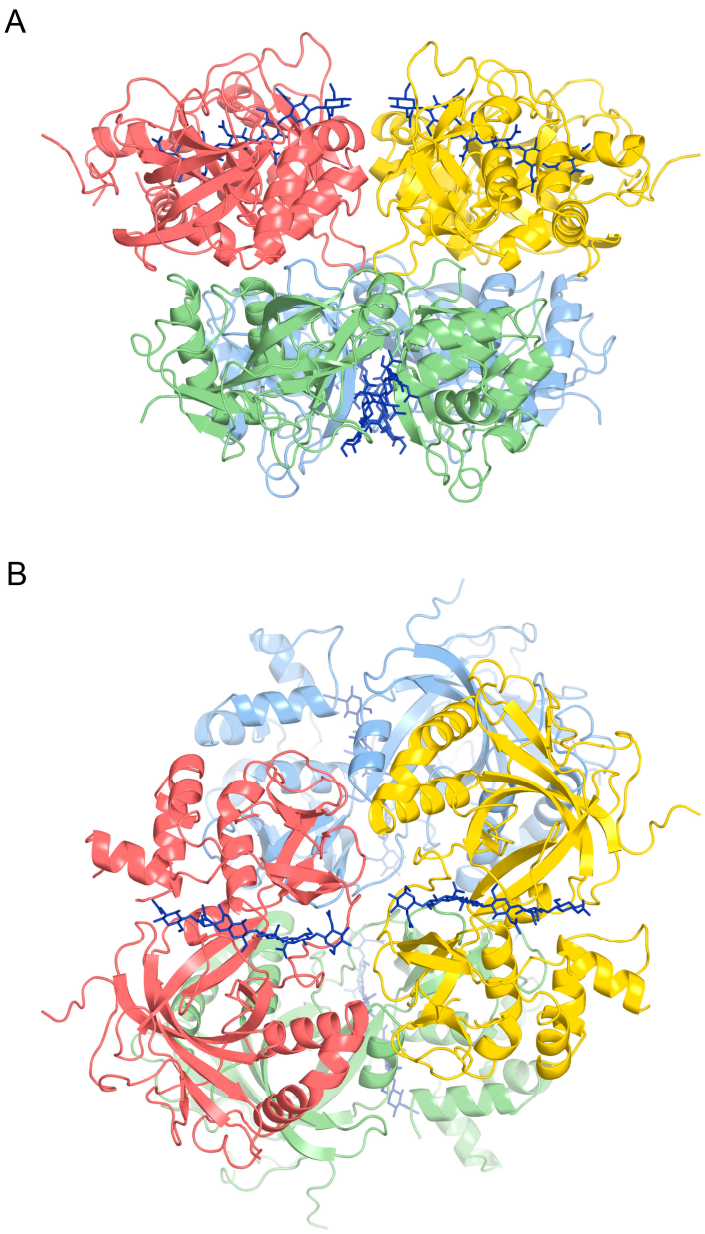

Figure S1. **The Crystallographic $(GlcNAc)_6$-Bound sMltA-D308A Tetramer.** *A,* Ribbon representation of the crystallographic $(GlcNAc)_6$-bound sMltA-D308A tetramer with the sMltA molecules in different colors. The hexasaccharide is shown in blue bonds. *B,* The view in (A) rotated by 90° along the horizontal axis.

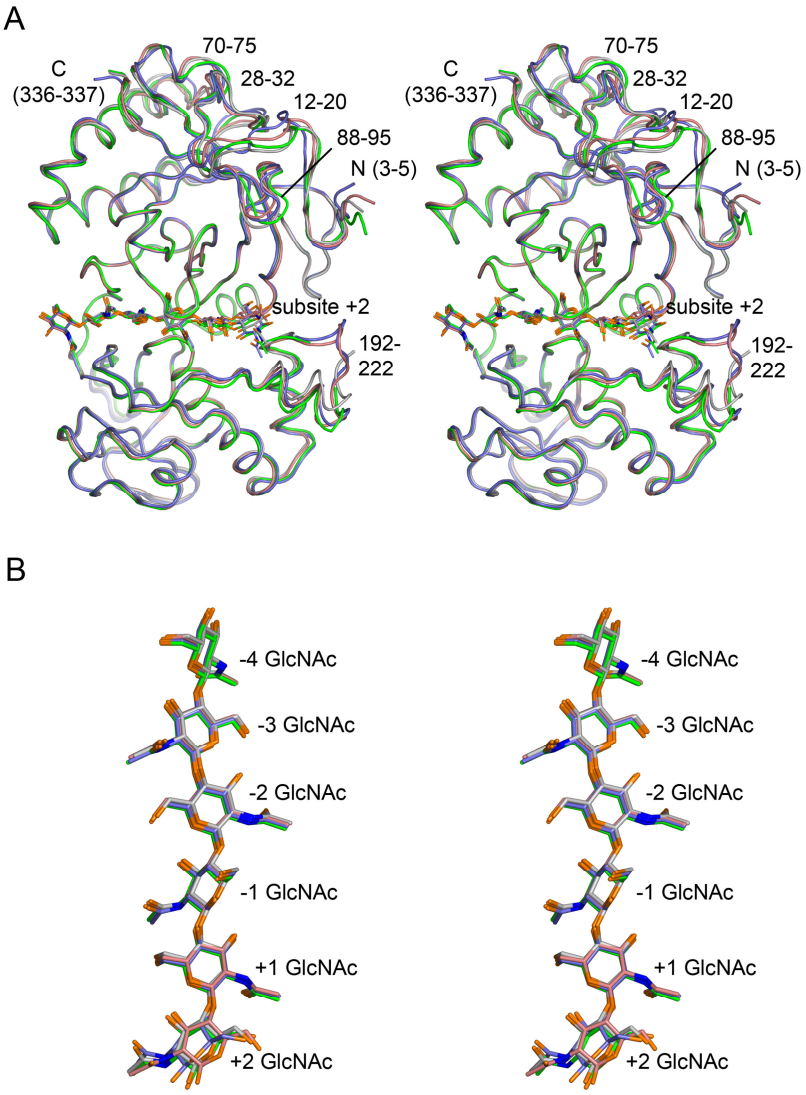

Figure S2. **Comparison of the Monomeric Subunit Structures in the Crystallographic $(GlcNAc)_6$-Bound sMltA-D308A Tetramer.** *A,* Stereo view showing a Cα-backbone superposition of the four, differently colored, $(GlcNAc)_6$-bound sMltA-D308A subunits. Regions in the structures which show substantial differences in conformation are indicated. *B,* Stereo view of an overlay of the four bound $(GlcNAc)_6$ in the tetramer, obtained by superimposition of the Cα-polypeptide backbones.

References

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